

## SHORT COMMUNICATION

# Mapping of the HSD17B2 Gene Encoding Type II 17 $\beta$ -Hydroxysteroid Dehydrogenase Close to D16S422 on Chromosome 16q24.1–q24.2

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The enzymes of the 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD) gene family are responsible for a key step in the formation and degradation of androgens and estrogens: catalyzing the interconversion of 17-ketosteroids and their active 17 $\beta$ -hydroxysteroid counterparts. The structure of human type II 17 $\beta$ -HSD cDNA was recently reported. This enzyme catalyzes the interconversion of  $\Delta^4$ -androstenedione and testosterone, androstenedione and dihydrotestosterone, and estrone and 17 $\beta$ -estradiol, whereas type I 17 $\beta$ -HSD catalyzes exclusively the interconversion of estrogens. To locate the HSD17B2 gene, the novel dinucleotide CA repeat sequence found 571 bp downstream from the end of exon 1 was genotyped into eight CEPH reference families by PCR. Two-point linkage analysis was performed between the latter polymorphism and the 2066 microsatellite markers of Généthon. The maximal pairwise lod score ( $Z_{\max} = 33.3$ ) with a maximal recombination fraction ( $\theta_{\max}$ ) of 0.008 was obtained with the marker D16S422 located on 16q24.1–q24.2. To define further the localization of the HSD17B2 gene, we constructed a high-resolution genetic map of the region flanking the polymorphic HSD17B2 gene including eight Généthon markers. The order of the HSD17B2 gene and markers is qter-D16S516 – D16S504 – D16S507 – D16S505 – D16S511 – [HSD17B2–D16S422]–D16S520–D16S413–tel. © 1995

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In humans, sex steroids are synthesized in large amounts in peripheral tissues from circulating precursors. The enzymes of the 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD) gene family are responsible for a key step in the biosynthesis and inactivation of androgens and estrogens: catalyzing the interconversion of 17-ketosteroids and their active 17 $\beta$ -hydroxysteroid counter-

parts. The human type I 17 $\beta$ -HSD gene (HSD17B1, previously designated EDH17B2; its suspected pseudogene is designated HSD17BP1) encodes a cytoplasmic protein of 327 amino acids expressed as a dimer catalyzing the interconversion of estrone and estradiol (8–10). The gene for HSD17B1 is closely linked with the susceptibility gene for hereditary breast-ovarian cancer, BRCA1, on the 17q21 region (13). More recently, type II 17 $\beta$ -HSD was isolated from a human prostate cDNA library, and the corresponding mRNA species was detectable in the placenta (15). This cDNA encodes a predicted protein of 387 amino acids with a molecular weight of 42,782, which is most probably associated with the membranes of the endoplasmic reticulum. This enzyme catalyzes the interconversion of estrone and 17 $\beta$ -estradiol,  $\Delta^4$ -androstenedione and testosterone, androstenedione and dihydrotestosterone, and 20 $\alpha$ -dihydroprogesterone and progesterone (15). This enzyme, chronologically designated type II 17 $\beta$ -HSD, is also a member of the short-chain alcohol dehydrogenase superfamily and shares only about 20% sequence identity with type I 17 $\beta$ -HSD.

To locate the HSD17B2 gene by genetic linkage mapping, we searched for DNA polymorphism within this gene. A dinucleotide CA repeat sequence was found 571 bp downstream from the end of exon 1. The sizes of the PCR fragments amplified with primers 5'-CTTGGC-ATCGTGTCCAACATTCAG-3' and 5'-CTTTCTGGG-TTAGGATAACGCTGG-3' were 215, 213, 211, 209, 207, and 205 for the alleles A1, A2, A3, A4, A5, and A6, respectively, which included 15 to 10 CA repeat sequences. Amplification was performed in a 20- $\mu$ l volume containing 8 ng of genomic DNA; 200  $\mu$ M each dCTP, dGTP, and dTTP; 4  $\mu$ M dATP; 100 nM [<sup>35</sup>S]-dATP; 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM MgCl<sub>2</sub>; 5% DMSO; 100 nM each primer; and 1.5 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus). After an initial denaturation at 100°C for 10 min, the samples were cooled to 72°C and processed through 35 cycles consisting of 1 min of denaturation at 95°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C using a Perkin-Elmer Cetus thermal cycler

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. L11708.

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TABLE 1

## Two-Point Linkage Analysis between HSD17B2 and Selected Markers on Chromosome 16

Locus	Lod scores at recombination fraction ( $\theta$ ) of:								$Z_{\max}$
	0.0	0.01	0.05	0.1	0.2	0.3	0.4	$\theta_{\max}$	
D16S516	$-\infty$	14.69	18.46	18.49	15.74	11.38	5.83	0.073	18.66
D16S504	$-\infty$	7.13	9.04	9.08	7.79	5.74	3.15	0.075	9.17
D16S507	$-\infty$	18.27	20.67	20.15	16.90	12.30	6.65	0.057	20.70
D16S505	$-\infty$	21.26	21.58	20.17	16.17	11.37	5.92	0.029	21.81
D16S511	$-\infty$	26.99	25.90	23.86	19.04	13.42	6.86	0.010	26.99
D16S422	$-\infty$	33.29	31.38	29.30	23.45	16.70	8.90	0.008	33.30
D16S520	$-\infty$	3.85	10.16	11.67	11.04	8.51	4.74	0.125	11.79
D16S413	$-\infty$	-0.79	8.74	11.40	11.54	9.06	5.01	0.149	11.95

(Model 480). The PCR products were resolved on a 5% polyacrylamide gel. The frequency of the 6 alleles observed, estimated from 100 chromosomes from 50 unrelated Caucasian individuals, were  $A1 = 0.04$ ,  $A2 = 0.04$ ,  $A3 = 0.26$ ,  $A4 = 0.03$ ,  $A5 = 0.10$ , and  $A6 = 0.53$ . The heterozygosity was 0.54.

The novel microsatellite marker in HSD17B2 gene was genotyped in the 8 largest reference CEPH families 102, 884, 1331, 1332, 1347, 1362, 1413, and 1416 (2), taking advantage of the existing framework maps of Généthon markers (3, 14). Two-point linkage analysis was performed between the polymorphism in HSD17B2 and the 2066 microsatellite markers of Généthon (3). Linkage analysis was performed using version 5.2 of the LINKAGE package of computer programs (5). Pairwise lod scores between the HSD17B2 6-allele polymorphism and the other markers were calculated using the MLINK program, whereas the  $\theta_{\max}$  and  $Z_{\max}$  were calculated using the CLODScore pro-

gram. The maximal pairwise lod score ( $Z_{\max} = 33.3$ ) with a maximal recombination fraction ( $\theta_{\max}$ ) of 0.008 was obtained with the marker D16S422 located on 16q24.1-q24.2 (Table 1).

To define further the localization of the HSD17B2 gene, we constructed a high-resolution genetic map of the region flanking the polymorphic HSD17B2 gene, including eight Généthon markers. The GMS program (generously provided by M. Lathrop) was used to calculate and validate the order of the framework markers (7). Multipoint linkage (CMAP program) was performed to determine the localization of HSD17B2 gene on the framework map. Genotyping error detection was performed by checking for double recombinants (6). The programs RTH and RECSTAT from M. Lathrop were used to haplotype the markers on CEPH families and count recombinants. The corrections eliminated all double recombinants, and a final GMS processing recalculated and revalidated the marker distances and orders. The HSD17B2 locus within the fixed map based on segregation analysis in the CEPH pedigrees is in the interval flanked by D16S511 and

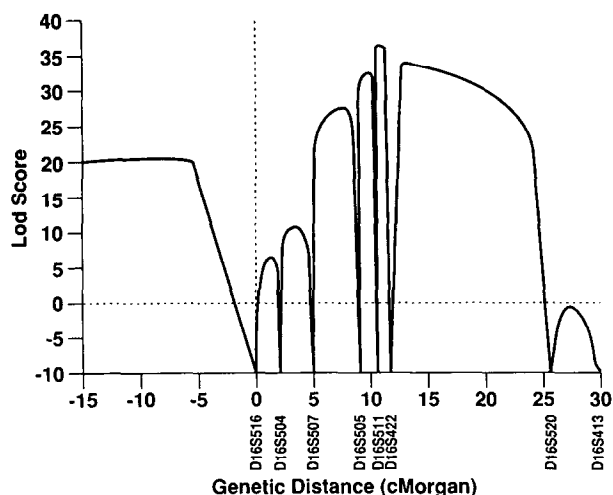


FIG. 1. Multipoint lod scores (base 10) for linkage of the locus HSD17B2 to the framework marker map calculated with CMAP. The curve indicates the likelihood that the HSD17B2 locus is at the map location with respect to adjacent markers, the positions of which are given by the vertical labels at the bottom of the x-axis. The recombination fractions between the framework markers were calculated using the CILINK program and converted to genetic distances using the Kosambi mapping function. The relative genetic position of D16S516 has arbitrarily been placed at zero.

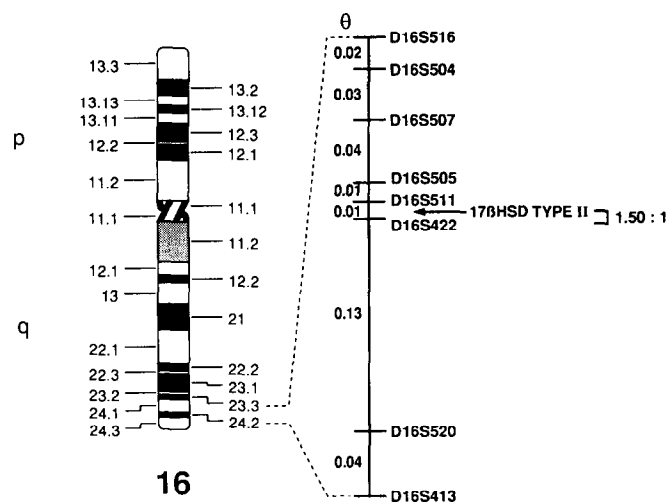


FIG. 2. Chromosome 16q24 area map. The map gives the best supported order of the markers and sex average recombination fractions ( $\theta$ ) between adjacent markers. Only D16S422 and HSD17B2 are ordered with odds <1000:1. The order of markers was determined as described (7).

D16S520 and could be located on either side of D16S422 (Fig. 1). The order of the HSD17B2 gene and selected markers is qter-D16S516- $2.18 \times 10^3$ -D16S504- $1.54 \times 10^5$ -D16S507- $1.18 \times 10^{10}$ -D16S505- $8.78 \times 10^4$ -D16S511- $7.62 \times 10^6$ -[HSD-17B2-1.50-D16S422]- $5.1 \times 10^{29}$ -D16S520- $1.97 \times 10^3$ -D16S413-tel. The most likely order is thus supported by odds of 1000:1 or greater against any permutation of two adjacent markers, except for HSD17B2 and D16S422, for which the odds are 1.50:1 (Fig. 2). The different localizations of the HSD17B1 and HSD17B2 genes are in agreement with their low sequence homology and suggest that they diverged very early in the evolution of the short-chain alcohol dehydrogenase superfamily. Moreover, mapping of the HSD17B2 gene to 16q24 will be useful for genetic analyses, especially knowing that a loss of heterozygosity has frequently been observed in this region in carcinomas of sex steroid target tissues including the prostate, endometrium, breast, and ovary (1, 4, 11, 12).

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#### REFERENCES

- Carter, B., Ewing, C., Ward, S., Treiger, B., Aalders, T., Schalken, J., Epstein, J., and Isaacs, W. (1990). Allelic loss of chromosome 16q and 10q in human prostate cancer. *Proc. Natl. Acad. Sci. USA* **87**: 8751-8755.
- Dausset, J., Cann, H., Cohen, D., Lathrop, M., Lalouel, J. M., and White, R. (1990). Centre d'étude du polymorphisme humain (CEPH): Collaborative genetic mapping of the human genome. *Genomics* **6**: 575-577.
- Gyapay, G., Morissette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M., and Weissenbach, J. (1994). The 1993-1994 Généthon human genetic linkage map. *Nature Genet.* **7**: 246-339.
- Kunimi, K., Bergerheim, U., Larsson, I., Ekman, P., and Collins, P. (1991). Allelotyping of human prostatic adenocarcinoma. *Genomics* **11**: 530-536.
- Lathrop, G. M., and Lalouel, J. M. (1984). Easy calculation of lod scores and genetic risk on small computers. *Am. J. Hum. Genet.* **36**: 460-465.
- Lathrop, G. M., and Lalouel, J. M. (1991). Statistical methods for linkage analysis. In "Handbook of statistics," Vol. 8, "Statistical Methods in Biological and Medical Sciences" (C. R. Rao and R. Chakraborty, Eds.), pp. 81-123, Elsevier, Amsterdam.
- Lathrop, M., Nakamura, Y., Cartwright, P., O'Connell, P., Lepert, M., Jones, C., Tateishi, H., Bragg, T., Lalouel, J. M., and White, R. (1988). A primary genetic map of markers on human chromosome 10. *Genomics* **2**: 157-164.
- Lin, S. X., Yang, F., Jin, J. Z., Breton, R., Zhu, D. W., Luu-The, V., Labrie, F. (1992). Subunit identity of the dimeric 17 $\beta$ -hydroxysteroid dehydrogenase from human placenta. *J. Biol. Chem.* **267**: 16182-16187.
- Luu-The, V., Labrie, C., Zhao, H.-F., Couët, J., Lachance, Y., Simard, J., Leblanc, G., Côté, J., Bérubé, D., Gagné, R., and Labrie, F. (1989). Characterization of cDNAs for human estradiol 17 $\beta$ -dehydrogenase and assignment of the gene to chromosome 17: Evidence of two mRNA species with distinct 5'-termini in human placenta. *Mol. Endocrinol.* **3**: 1301-1309.
- Luu-The, V., Labrie, C., Simard, J., Lachance, Y., Zhao, H.-F., Couët, J., Leblanc, G., and Labrie, F. (1990). Structure of two in tandem human 17 $\beta$ -hydroxysteroid dehydrogenase genes. *Mol. Endocrinol.* **4**: 268-275.
- Risinger, J., Berchuck, A., Kohler, M., and Boyd, J. (1994). Mutations of the E-cadherin gene in human gynecologic cancers. *Nature Genet.* **7**: 98-102.
- Sato, T., Saito, H., Morita, R., Koi, S., Lee, J., and Nakamura, Y. (1991). Allelotype of human ovarian cancer. *Cancer Res.* **51**: 5118-5122.
- Simard, J., Feunteun, J., Lenoir, G., Tonin, P., Normand, T., Luu-The, V., Vivier, A., Lasko, D., Morgan, K., Rouleau, G., Lynch, H., Labrie, F., and Narod, S. (1993). Genetic mapping of the breast-ovarian cancer syndrome to a small interval on chromosome 17q12-21: Exclusion of candidate genes EDH17B2 and RARA. *Hum. Mol. Genet.* **2**: 1193-1199.
- Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., Millasseau, P., Vaysseix, G., and Lathrop, M. (1992). A second-generation linkage map of the human genome. *Nature* **359**: 794-801.
- Wu, L., Einstein, M., Geissler, W., Chan, K., Elliston, K., and Andersson, S. (1993). Expression cloning and characterization of human 17 $\beta$ -hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20 $\alpha$ -hydroxysteroid dehydrogenase activity. *J. Biol. Chem.* **268**: 12964-12969.